

GENE REGULATION AND ONCOGENESIS BY RETROVIRUSES. Harold E. Varmus, Department of Microbiology & Immunology, University of California, San Francisco, CA 94143.

Retroviral DNA is equipped with long terminal repeats (LTRs) that can influence expression of both viral and cellular genes. I will review our several efforts to define the biochemical basis and biological consequences of these regulatory events.

(1) Determination of the nucleotide sequences of LTRs reveals signals and sites believed to affect initiation and polyadenylation of viral RNAs.

(2) The functional capacities of LTRs have been studied by construction of recombinant plasmids, containing LTRs and cloned genes in various arrangements, followed by their introduction into cultured cells. As expected, only an LTR in the correct transcriptional orientation upstream from a promoter-less gene can produce expression of that gene. However, when the gene carries its own promoter, the efficiency of DNA transformation is enhanced many-fold by the presence, in either orientation, of certain LTRs (e.g. that of Rous sarcoma virus (RSV)), suggesting that some LTRs may enhance gene expression at distant, heterologous promoters in a non-polar fashion. The LTR of mouse mammary tumor virus (MMTV) governs expression of viral or linked cellular genes in a glucocorticoid-responsive manner; the region responsible for hormonal regulation has been localized to ca. 0.4 kb near the 3' end of the MMTV LTR.

(3) The induction of B-cell lymphomas by avian leukosis virus (ALV) appears to require insertion of proviral DNA near c-myc, a putative cellular oncogene. The viral inserts, sometimes comprising little more than a single LTR, can enhance expression of c-myc in at least three arrangements: when positioned upstream from c-myc in the same transcriptional orientation, the inserts serve as promoters for transcription of c-myc; when positioned upstream in the opposite orientation to c-myc or downstream in the same orientation, they enhance expression from unidentified cellular promoter(s).

In the last instance, the LTR provides a novel site for polyadenylation. Interrupted c-myc loci have been cloned from tumor DNA and reintroduced into cultured cells to identify determinants of the enhancement phenomena.

(4) MMTV, like ALV, lacks a viral oncogene, yet regularly induces tumors. By cloning the integration site from a mammary carcinoma bearing a single MMTV provirus, we have identified a 20 kb region of the mouse genome in which proviruses are frequently found in tumors. Adjacent transcriptional domains that may be affected by these insertions are currently being sought.

(5) The activity of LTRs appears to be influenced by each other and their chromosomal context. To understand these influences, we are exploring the following situations: (a) a murine leukemia virus LTR, placed within a transcriptionally-active RSV provirus by infection, does not polyadenylate or initiate transcripts; (b) ostensibly identical RSV proviruses at different chromosomal sites display differential stability of gene expression; (c) expression of one such RSV provirus is successively diminished and enhanced at high frequency; (d) expression of another RSV provirus is affected only by rare spontaneous deletions which remove the 5' LTR.